Optical Trapping and Single Molecule Fluorescence Lab Demonstration
Dual Confirmation of double-strand DNA Unzipping

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Summary: Optical tweeezers are an excellent experiemental tool to study the biophysics of single molecules including molecular motors (kinesin, myosin, RNA polymerase), mechanical conformations/transitions of molecules (dsDNA, RNA hairpins, filamentous proteins) and receptor-ligand interactions (anitgen-antibody). In the most common assays, the mechanical state of the system is monitored by tracking the position of a handle (usually a dielectric microsphere with diameter of 0.5-2um) tethered to the subject of interest (protein, DNA, etc), with nanometer resolution. The handle also serves as probe to apply force to the system to study the energetics of mechanical changes. Single molecule fluorescence allows the direct observation of the mechanical/conformational changes of the system as it is subjected to perturbations, such as force. The combination of these two techniques allows researches to study the biophysical properties of single molecules. In this lab you will learn the basics of operating a high-end optical tweezers to record mechanical transitions of single molecules. The instrument is also equipped with a novel single molecule fluorescence technique to allow simultaneous, coincident optical trapping and single molecule flourescence. In our demonstration we will measure the force required to unzip a double-stranded DNA molecule, with a resolution of ~5nm and ~0.1pN, while using single molecule fluorescence to confirm the location of the break. Alignment and calibration procedures will be also presented.

Recommended Reading

R.R. Brau *et al*, "Interlaced Optical Force-Fluorescence Measurements for Single Molecule Biophysics," *Biophys. J.* **91** (2006).

M.J. Lang *et al*, "Combined Optical Trapping and Single-Molecule Fluorescence," *J. Biol.* **2** (2003).

(optional) Optical Trapping Review : K.C. Neuman & S.M. Block, "Optical trapping," *Rev. Sci. Instrum.* **75** (2003).

Equipment you will see in the Lang Lab:

- □ Modified Nikon TE2000 microscope (100X, 1.40 oil immersion objective)
  - o Piezo-electric stage (1-nm resolution, 100x100x20 μm range)
  - o Manual stage
  - o Position detection branch with photodiode
- □ Main optics box (right of microscope): enclosed breadboard housing the trapping (1064nm), detection (975nm) and fluorescence excitation (488nm and 532nm) lasers, and optics to align the beams. A pair of acousto-optic deflectors (AODs) provides 2-D control of the trapping laser location in the specimen plane and a single AOD provides modulation of the fluorescence excitation laser.
- □ Dark box (left of microscope): contains a CCD for bright field imaging, an intensified CCD for single molecule imaging and a pair of silicon avalanche photodiodes (SAPDs) for single molecule detection and fluorescence resonance energy transfer (FRET) detection.
- □ All of the above mounted on top of an optical table.

Experimental procedure for single molecule fluorescence using total internal reflection fluorescence (TIRF) microscopy:

- □ Set up slide in scope, adjust bright field illumination and position detection branch (attached to condenser).
- □ Locate single dye on surface using intensified CCD.
- □ Move the molecule to the fluorescence collection region (pinhole) using piezo-stage.
- □ Collect fluorescence emission with SAPDs (counts per unit of time) until dye photobleaches (discrete reduction in fluorescence signal).

Combined OT and SMF to detect dsDNA unzipping:

- □ Find a tethered bead, find the tether point and reposition it to the fluorescence collection region.
- □ Trap the bead and simultaneously record bead displacement from the center of the trap and fluorescence emission.
- □ An unzipping event will show both a sudden change in bead position and a discrete decrease in fluorescence emision (dye no longer in collection region).
- □ Calibrate the bead for both position and obtain trap stiffness using variance method (see review by Neuman & Block for details).